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Award Number: DAMD17-99-1-9125

TITLE: Functional Imaging of Multidrug Resistance in Breast Cancer with PET

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REPORT DATE: August 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 2002	Annual (1 Aug 01 - 31 Jul 02)

4. TITLE AND SUBTITLE
Functional Imaging of Multidrug Resistance in Breast Cancer with PET

5. FUNDING NUMBERS
DAMD17-99-1-9125

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*
The objective of this project is the development of a PET radiopharmaceutical for measuring multidrug resistance (mdr) in breast cancer. Multidrug resistance is resistance of a lesion to a specific class of drugs that includes many of the chemotherapeutics that are most effective against breast cancer. Single-photon myocardial perfusion agents such as ^{99m}Tc-MIBI are substrates for Pgp, the protein implicated in mdr, and are now being studied for evaluation of mdr. A PET mdr tracer would provide significant advantages over ^{99m}Tc-MIBI, and the half-life of ^{64}Cu (12.7 h) is better matched to the apparent biological half-life of the mdr process in breast cancer (~240 min.) than are other PET radionuclides (e.g., ^{11}C, $T_{1/2}$= 11 min). We are carrying out *in vivo* and *in vitro* studies of lipophilic cationic ^{64}Cu-based PET radiopharmaceuticals as potential PET mdr radiopharmaceuticals using murine (MAT-B) and human (MCF-7) breast cancer models. *In vitro* studies of prototype ^{64}Cu complexes reveal a pattern of uptake similar to that observed for ^{99m}Tc-MIBI. Studies are currently underway to determine the optimal chemical properties of this agent. The development of a radiopharmaceutical for the measurement of the mdr status of breast cancer lesions will facilitate optimization of treatment protocols, monitoring of the development of acquired resistance, and real-time evaluation of mdr modulators.

14. SUBJECT TERMS
breast cancer, PET, ^{64}Cu, multidrug resistance

15. NUMBER OF PAGES
12

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

INTRODUCTION

The objective of this project is the development of a PET (Positron Emission Tomography) radiopharmaceutical for the quantitative functional evaluation of multidrug resistance (mdr) in breast cancer. Multidrug resistance (mdr) is defined as intrinsic or acquired resistance to a specific class of chemotherapeutic drugs, which includes many of the most effective chemotherapeutic agents against breast cancer.

Multidrug resistance is characterized by overexpression of the *MDR1* and *MRP* genes and increased concentrations of P-glycoprotein (Pgp), a 170 kD transmembrane glycoprotein, and multidrug-resistance protein (MRP1), a 190 kD protein, which reduce the intracellular concentration of the drugs to non-toxic levels. Lipophilic cationic complexes such as ^{99m}Tc-MIBI are substrates for Pgp and MRP1 and are now being studied for clinical evaluation of mdr. We are carrying out *in vivo* and *in vitro* studies of lipophilic cationic ^{64}Cu-based PET radiopharmaceuticals derived from copper-diiminedioxime complexes (Fig. 1) as possible PET mdr radiopharmaceuticals.

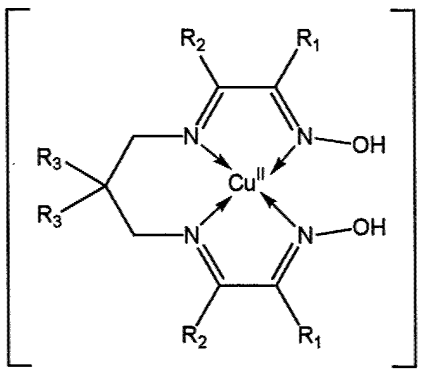


Fig. 1. Copper(II) Diiminedioxime (PreH) Complex

A PET mdr tracer will provide significant advantages over ^{99m}Tc-MIBI (e.g., straightforward attenuation corrections, higher spatial resolution, greater sensitivity, and the ability to perform quantitative studies), and the half-life of ^{64}Cu (12.7 h) is better matched to the apparent biological half-life of the mdr process (~240 min.) than are other PET radionuclides such as ^{11}C ($T_{1/2}$= 11 min). Furthermore, ^{11}C-based radiopharmaceuticals are only available at a limited number of institutions and, because of the short half-life of ^{11}C, cannot be shipped to other institutions. This new radiopharmaceutical will provide real-time information about the mdr status of breast cancer lesions that may allow optimization of treatment protocols, monitoring of the development of acquired resistance, and evaluation of the effectiveness of drugs developed to modulate mdr.

BODY

The research accomplishments are discussed in terms of each Task outlined in the Revised Statement of Work (6/1/99).

Task 1: Recruitment and training of research technician, Months 1-3

Mr. Robert Borgesi continues to fill the position of Research Technician on this project.

Task 1 has been completed.

Task 2: Establish and validate *in vitro* assay for multidrug resistant breast cancer, Months 3-12

a. Establish breast cancer cell lines
b. Establish mdr breast cancer cell lines
c. Validate parental and mdr breast cancer cell lines with ^{99m}Tc-MIBI
d. Validate parental and mdr breast cancer cell lines with prototype ^{64}Cu PreH and cyclops complexes

Two breast cancer cell lines, the rat MAT-B and the human MCF-7, and their drug resistant sub-lines were established previously in our laboratory.

We previously reported the validation of these cell lines in our laboratory with ^{99m}Tc-MIBI and [^{64}Cu(Me_2MAKPreH)]$^+$) (Fig. 1, R_1 = -$(CH_2)_3CH_3$, R_2 = -CH_3, R_3 = -CH_3)

Task 2 has been completed.

Task 3: Establish and validate *in vivo* assay for multidrug resistant breast cancer, Months 3-12

a. Establish breast cancer cell lines in animal model
b. Establish mdr breast cancer cell lines in animal model
c. Validate parental and mdr breast cancer cell lines with ^{99m}Tc-MIBI
d. Validate parental and mdr breast cancer cell lines with prototype ^{64}Cu PreH and cyclops complexes

The parental and resistant MAT-B and MCF-7 breast cancer cell lines were previously established.

The MAT-B cell line was established in the Sprague-Dawley rat after an amendment to the animal protocol was submitted and approved that allows us to carry out studies using the parental and drug-resistant MAT-B cell lines simultaneously in the same animal. No *in vivo* studies have been undertaken to date with the MCF-7 cell line because at this point the additional information that may be gained by using the human cell line is not considered to offset the significantly higher cost of the nude mice required for these studies.

An *in vivo* study using the MAT-B cell lines and the prototype ^{64}Cu complex were carried out in Sprague-Dawley rats. The non-resistant tumor was implanted in the right thigh (10^6 cells) and the drug resistant tumor was implanted in the left thigh (10^6 cells). The tumors were allowed to grow for approximately two weeks at which point the biodistribution study was carried out. Each animal was injected with 25 μCi [^{64}Cu(Me_2MAKPreH)]$^+$ and sacrificed at selected time intervals post injection. The results of this study are summarized below (Table 1).

Table 1. Biodistribution of [^{64}Cu(Me_2MAKPreH)]$^+$ (% i.d./g, n=5)

Tissue	Time Post-Injection (min.)		
	5	15	60
Blood	0.41 ± 0.05	0.29 ± 0.06	0.36 ± 0.02
Heart	1.55 ± 0.11	1.10 ± 0.14	0.92 ± 0.11
Lungs	1.45 ± 0.11	0.75 ± 0.14	0.54 ± 0.04
Liver (sample)	6.26 ± 0.76	5.26 ± 0.98	1.55 ± 0.23
Spleen	2.15 ± 0.40	1.64 ± 0.37	0.56 ± 0.07
Kidneys	10.26 ± 1.37	8.48 ± 1.67	3.90 ± 0.75
Gut	0.52 ± 0.16	0.52 ± 0.25	0.79 ± 0.49
Brain	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.00
Skin/Fur/Fat	0.22 ± 0.06	0.28 ± 0.06	0.31 ± 0.04
Muscle	0.23 ± 0.03	0.24 ± 0.04	0.28 ± 0.05
Bone	0.40 ± 0.05	0.37 ± 0.08	0.32 ± 0.06
Tumor (resistant)	0.21 ± 0.04	0.21 ± 0.05	0.46 ± 0.19
Tumor (non-resistant)	0.30 ± 0.04	0.30 ± 0.06	0.38 ± 0.08

The most interesting observation from the point of view of this project is that at 5 and 15 min. post-injection, the uptake of ^{64}Cu is lower in the resistant than in the non-resistant tumors (P<0.05). This is the result that would be expected if the ^{64}Cu complex is a substrate for Pgp. There is, however, no difference between the resistant and non-resistant tumors at the 60 min. time point (P>0.10). The pattern of uptake is similar to that observed with ^{99m}Tc-MIBI except for

the increase in uptake by the resistant lesion at 60 min. post-injection, which is marginally significant (P=0.05).

It is also significant that the amount of tracer found in the heart in this study is lower than was observed in previous studies with this complex (3.0-3.5% i.d./g). The earlier studies were not, however, carried out at the no-carrier-added level, which raises the possibility that there may be some decomposition of the complex *in vivo*. We are currently exploring this possibility in several ways. First, we will repeat the biodistribution experiment and include a single animal that is injected with a larger amount of tracer. A blood sample will be obtained from this animal and analyzed to determine the chemical form of the tracer that is present in the circulation. Second, we are repeating the *in vitro* studies with this compound to determine if addition of the non-radioactive complex, which would inhibit decomposition, increases uptake in the MAT-B cells. Third, we have developed a rapid, high-yield synthesis for the "closed" (cyclops) complex in which the hydrogen bond between the two oxime moieties is replaced by a covalent BF_2 linkage. This complex is expected to be more stable *in vivo* because it the macrocycle is closed by a "real" macrocycle (i.e., one closed by a covalent bond) as opposed to the pseudo-macrocycle (i.e., one closed by a hydrogen bond) in the PreH complex. An *in vitro* study will be carried out with this compound in the near future. If this study shows significant differences between the two compounds, an *in vivo* study will be carried out as described above.

Additional *in vivo* studies will be carried out as we identify, based on the *in vitro* studies, ^{64}Cu complexes that have higher cell uptake or greater resistant/parental differentiation than the prototype complex, $[Cu(Me_2MAKPreH)]^+$.

An additional aspect of the validation of the MAT-B and MCF-7 cells lines is measurement of Pgp in the parental and resistant lines. This measurement is carried out by Western Blot assay using the C219 antibody. The method was validated using the parental and drug-resistant MES-SA cell lines because the Pgp expression in these cell lines is well known. The Western Blot assay confirmed that the Pgp concentration in the resistant sub-line was higher than in the parental line. We are now carrying out this assay on the parental and resistant breast cancer cell lines.

Parts a and b of this task have been completed. Parts c and d are on-going.

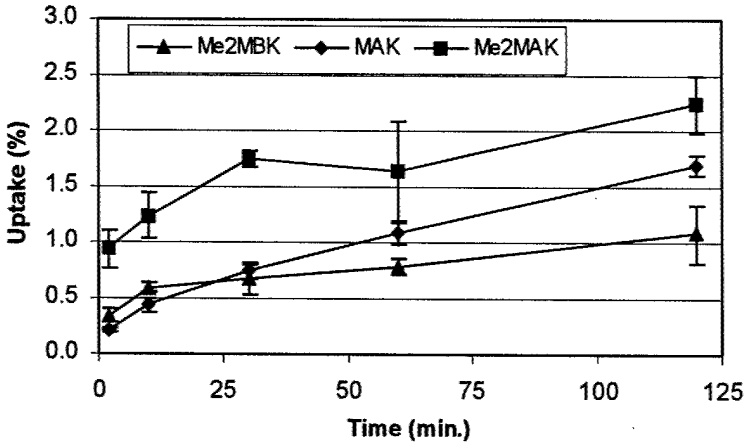
Task 4: Evaluate the effect of chemical properties (lipophilicity, ligand geometry, ligand substituents) on the biological properties of a copper-based PET radiopharmaceutical for the functional assessment of multidrug resistance in breast cancer, Months 13-24
- a. *In vitro* studies of the ^{64}Cu-labeled complexes using breast cancer model
- b. *In vivo* studies of the ^{64}Cu-labeled complexes using breast cancer model

In the past year we carried out *in vitro* studies on two ^{64}Cu diiminedioxime complexes that are chemically similar to $[Cu(Me_2MAKPreH)]^+$. $[Cu(Me_2MAKPreH)]^+$ was chosen as the starting point because its uptake was clearly superior to that of less lipophilic complexes with the MES-SA cell line. The two ligands were $Me_2MBKPreH$ ($R_1 = (CH_2)_2CH_3$, $R_2 = CH_3$, $R_3 = CH_3$) and MAKPreH ($R_1 = (CH_2)_3CH_3$, $R_2 = CH_3$, $R_3 = H$), which each have two fewer carbon atoms than $Me_2MAKPreH$. These studies were carried out using the MCF-7 cell line. For clarity, only the results for the parental line without Cyclosporin A are shown in Figure 2.

This graph shows that, as expected, the uptake of the complexes by the MCF-7 cells is lower for the both compounds as would be predicted for complexes that are less lipophilic than $[Cu(Me_2MAKPreH)]^+$. There is perhaps a small difference between the two less lipophilic complexes, which have similar lipophilicity but different geometry. Other results (not shown) for the resistant cells and the effect of Cyclosporin A follow the pattern previously reported for $[Cu(Me_2MAKPreH)]^+$ in other cell lines. This experiment will be repeated with the MAT-B cell

lines, with complexes that have the same number of carbon atoms as $[Cu(Me_2MAKPreH)]^+$ but different geometry (e.g., $[Cu(MHKPreH)]^+$, ($R_1 = (CH_2)_4CH_3$, $R_2 = CH_3$, $R_3 = H$), and with complexes that contain two more carbon atoms than $[Cu(Me_2MAKPreH)]^+$ (e.g., $[Cu(Me_2MHKPreH)]^+$, ($R_1 = (CH_2)_4CH_3$, $R_2 = R_3 = CH_3$) to evaluate the differences between the two cell lines and the effects of ligand geometry, if any.

Figure 2. Comparison of Uptake of Several Diiminedioxime Derivatives in MCF-7 Cells.

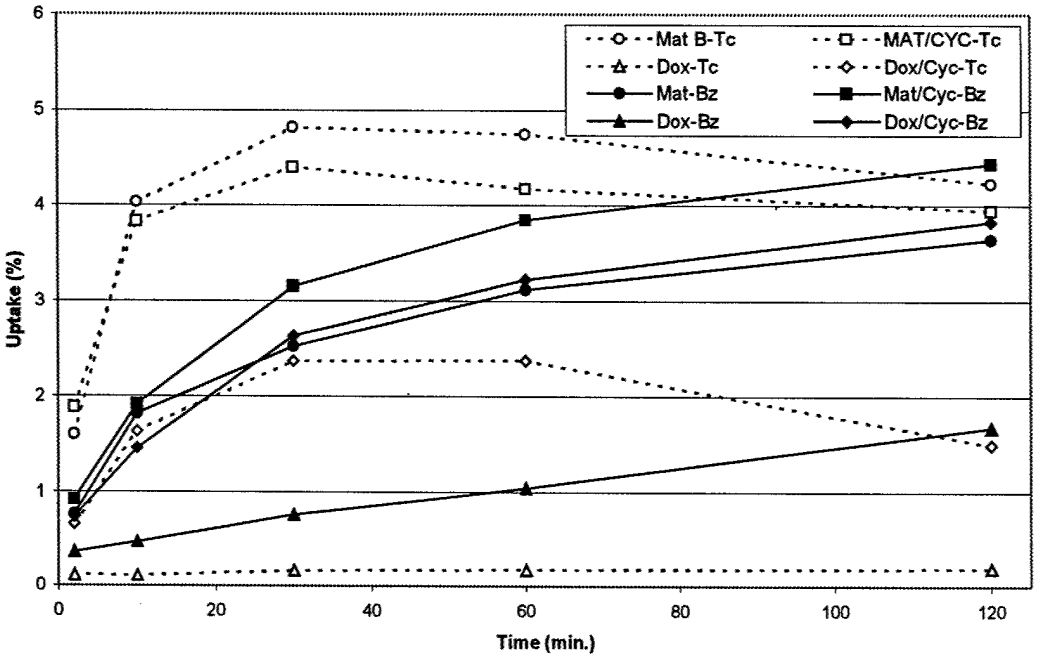


The current focus of our investigation is the introduction of methoxy or ethoxy substituents into the ligand. Previous studies of other radiopharmaceuticals that are also substrates for Pgp have shown that in all cases inclusion of these functional groups improves the biological properties of the complex. Our original effort was directed towards the use of alkyl ether substituents at the R_1 position, but we encountered significant problems in the synthesis of ligands including these moieties. To circumvent this problem, we are now preparing derivatives that include aryl ethers at the R_1 position (e.g., 4-methoxybenzyl). As the first step in this direction, we prepared the benzyl derivative, $[^{64}Cu(benzylPreH)]^+$ ($R_1 = -CH_2-C_6H_5$, $R_2 = CH_3$, $R_3 = H$) and measured its uptake in the MAT-B cell line. The results of this study are summarized in Figure 3, which compares the cell uptake of the benzyl complex to that of ^{99m}Tc-MIBI.

In this graph, the ^{99m}Tc-MIBI data is shown with dotted lines, and the ^{64}Cu-benzylPreH data is shown with solid lines. The parental MAT-B data are shown with circles (● - ^{64}Cu, ○ - ^{99m}Tc), the parental MAT-B data with Cyclosporin A are shown with squares (■ - ^{64}Cu, □ - ^{99m}Tc), the resistant MAT-B data without Cyclosporin A are shown with triangles (▼ - ^{64}Cu, ▽ - ^{99m}Tc, and the resistant MAT-B data with Cyclosporin A are shown with diamonds (♦ - ^{64}Cu, ◊ - ^{99m}Tc).

These data show that the uptake of this PreH derivative is 65% that of ^{99m}Tc-MIBI at 60 min (3.1% vs. 4.7%) and more than 85% that of ^{99m}Tc-MIBI at 120 min. (3.6% vs. 4.2%) This result is the closest to ^{99m}Tc-MIBI that we have achieved to date. In comparison to the prototype ^{64}Cu complex, the cell uptake of the benzyl complex is more than twice that of $[^{64}Cu(Me_2MAK)]^+$ at both 60 (3.1% vs. 1.3%) and 120 min. (3.6% vs. 1.6%). At all time points, it shows the expected pattern of uptake by the resistant and non-resistant cells as well as the expected response to Cyclosporin A. This result is especially encouraging because the study of the benzyl complex was only carried out as a baseline for comparison with the 4-methoxybenzyl derivative, which is currently being synthesized.

Figure 3. Comparison of Uptake of ^{99m}Tc-MIBI and [^{64}Cu(benzylPreH)]$^{+}$ by MAT-B Cells



We have also initiated studies aimed at determining the mechanism of uptake of the ^{64}Cu complexes by breast cancer cells. These cells are patterned after similar studies with ^{99m}Tc-MIBI in chick myocytes and fibroblasts (Chiu, et al., 1990, Piwnica-Worms, et al., 1990). In summary, the uptake of the ^{64}Cu complexes by tumor cells will be measured in the presence and absence of various metabolic inhibitors. These compounds include valinomycin, which depolarizes the mitochondrial membrane, nigericin, which hyperpolarizes the mitochondrial membrane, and high K buffer, which depolarizes the cell membrane. We are now carrying out the studies using ^{99m}Tc-MIBI both to validate the methodology and to determine baseline values with a known Pgp substrate in the breast cancer cell lines. We anticipate that studies with ^{64}Cu complexes will be undertaken in the next three to six months.

We will continue to test only the most promising complexes *in vivo*. As previously described, these studies will use two separate animal models. The primary model will be the MAT-B (rat) model which was used in the biodistribution study of [Cu(Me_2MAKPreH)]$^{+}$ described above. This model will be used for standard biodistribution studies. These have been initiated and will continue as promising new complexes are identified in the *in vitro* studies. Additional studies will be carried out using nude mice bearing MCF-7 tumors as indicated by the results of the studies with the MAT-B model.

Parts a and b of this Task are on-going.

Task 5: Evaluate differences between biological properties of the ^{64}Cu PET radiopharmaceuticals in breast and non-breast mdr tumor models, Months 13-24

The uptake of [^{64}Cu(Me_2MAKPreH)]$^{+}$ in the parental and resistant breast cancer cell lines, MCF-7 and MAT-B, was compared to the uptake in the parental and resistant MES-SA cell lines. This comparison revealed a similar pattern for all three types of cells. In each case, a maximum uptake of approximately 2% was observed for the parental cells after 2 hours. This compares to 0.2% - 1.2% for the resistant lines. For all three cell lines, addition of Cyclosporin A significantly increased uptake of the tracer by the cells.

This Task has been completed for the $[^{64}Cu(Me_2MAKPreH)]^+$ and will be repeated as necessary as promising complexes are identified.

Task 6: Integrate results of Task 4 and Task 5 into the development of a ^{64}Cu-based PET radiopharmaceutical for the evaluation of mdr in breast cancer, Months 13-36.

This Task will continue throughout the project.

KEY RESEARCH ACCOMPLISHMENTS

- *In vivo* validation of parental and drug-resistant MAT-B (rat) breast cancer cell lines with prototype ^{64}Cu-$Me_2MAKPreH$ complex
- Western blot assay of Pgp in parental and resistant cells validated with MES-SA cells
- *In vitro* comparison of effect of variation in substituent position on ligand on uptake by parental and resistant MCF-7 cells.
- *In vitro* studies of benzyl diiminedioxime derivative that show cell uptake approaching that observed for ^{99m}Tc-MIBI.
- Preliminary studies of mechanism of uptake using metabolic promoters/inhibitors in breast cancer cell lines
- Comparison of uptake of $[^{64}Cu(Me_2MAKPreH)]^+$ between the MAT-B and MCF-7 breast cancer cell lines and MES-SA cell lines

REPORTABLE OUTCOMES

Abstracts and presentations (A copy of this abstract is included in the Appendix)

1. Packard, AB, Kiani S, Borgesi R, Barbarics E. "Development of a ^{64}Cu-based PET Radiopharmaceutical for imaging MDR." Era of Hope, Orlando, FL, September, 2002.

Cell lines (previously reported)

The MES-SA and MES-SA/Dx5 (human uterine sarcoma) cell lines are established and being used for method validation.

The parental and multidrug-resistant MAT-B (rat mammary adenocarcinoma) cell lines are established and validated.

The parental and multidrug-resistant MCF-7 (human mammary adenocarcinoma) cell lines are established and validated.

CONCLUSIONS

After some delays in year-01, we are now using the MAT-B (rat) and MCF-7 (human) breast cancer cell lines *in vivo* and *in vitro* to evaluate new ^{64}Cu complexes. We have also undertaken preliminary studies to investigate the mechanism of uptake of these compounds. As part of our effort to prepare an ether-functionalized PreH derivative, we prepared a benzyl derivative which shows 2-3 times higher cell uptake than the prototype ^{64}Cu complex, $[Cu(Me_2MAKPreH)]^+$. WE also compared the uptake of $[Cu(Me_2MAKPreH)]^+$ in three cell lines, and observed that the total accumulation, the pattern of uptake in the resistant and parental cells, and the response to Cyclosporin A is similar for all three. We also observed that there is a difference in uptake between resistant and non-resistant MAT-B tumors *in vivo*.

Although these results are preliminary, they demonstrate that the pattern of uptake of this complex parallels the drug resistant status of the tumor cells, which supports our hypothesis that

these complexes may prove useful as PET radiopharmaceuticals for the evaluation of mdr in breast cancer. Additional *in vitro* studies are carried out biweekly. These results are being added to a database that will be used to develop structure/biodistribution relationships that will guide the development of new copper complexes. The *in vivo* evaluation of the first ^{64}Cu complexes will be carried out within the next six to eight weeks. These data will also be added to the database as they are accumulated.

The project is now on schedule to complete the evaluation of the ^{64}Cu complexes as originally scheduled.

REFERENCES

Chiu, M. L., J. F. Kronauge, et al. (1990). "Effect of mitochondrial and plasma membrane potentials on accumulation of hexakis (2-methoxyisobutylisonitrile) technetium(I) in cultured mouse fibroblasts." Journal of Nuclear Medicine **31**: 1646-53.

Piwnica-Worms, D., J. F. Kronauge, et al. (1990). "Uptake and retention of hexakis (2-methoxyisobutyl isonitrile) technetium(I) in cultured chick myocardial cells. Mitochondrial and plasma membrane potential dependence." Circulation **82**: 1826-38.

APPENDIX

Abstract

1. Packard, AB, Kiani S, Borgesi R, Barbarics E. "Development of a ^{64}Cu-based PET Radiopharmaceutical for imaging MDR." Era of Hope, Orlando, FL, September, 2002.

DEVELOPMENT OF A ^{64}Cu-BASED PET RADIOPHARMACEUTICAL FOR IMAGING MDR

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Multidrug resistance (MDR) is a treatment-limiting phenomenon frequently encountered during chemotherapy in which a malignancy fails to respond to or becomes resistant to a specific class of chemotherapeutics that includes many of the chemotherapeutic agent that are most effective against breast cancer (e.g., doxorubicin, daunorubicin, paclitaxel). It is characterized by increased concentrations of P-glycoprotein (Pgp) and multidrug-resistance protein (MRP1), which reduce the concentration of these chemotherapeutic agents in the cancer cells. Although well characterized in the laboratory, it is difficult to evaluate multidrug resistance in patients. If this information was available, it could be used to optimize treatment protocols, monitor the development of acquired resistance, and evaluate the effectiveness of MDR modulators.

The objective of this project is to develop a PET (Positron Emission Tomography) radiopharmaceutical for the functional evaluation of MDR. Lipophilic cationic ^{99m}Tc radiopharmaceuticals are known to be substrates for Pgp and MRP1. On this basis, we are developing a lipophilic cationic ^{64}Cu radiopharmaceutical that is a substrate for Pgp and/or MRP1, which can be used for functional imaging of MDR with PET. The basis for the development of this agent is copper complexes of a class of ligands known as diiminedioximes, which can be readily modified to optimize their biological properties.

The uptake of the prototype ^{64}Cu complex by parental and drug resistant MAT-B (rat) and MCF-7 (human) breast cancer cells was measured by incubating a solution of the complex with an equal volume of cell suspension at 37°C, removing samples from the suspension at selected time intervals, and measuring the activity in the cells and the media. The ability of the ^{64}Cu complexes to monitor Pgp expression in the cell lines was measured by repeating this procedure with 10 μM Cyclosporin A added to the incubation media.

The maximum uptake of the ^{64}Cu complex by both cell lines was 1.5% and still increasing at 120 min. At 120 min., uptake of ^{64}Cu by the parental MCF-7 cells is more than twice that of resistant cells. For MAT-B cells, the parental/resistant uptake ratio is approx. 8. In both cases addition of Cyclosporin A increased the uptake: for MAT-B cells to approx. one-half that of the parental cells, for MCF-7 to the same as that of the parental cells. Cyclosporin A had little or no effect on uptake by either parental cell line. For both cell lines, the maximum uptake was approx. 1/3 of that observed for ^{99m}Tc-MIBI.

These results demonstrate that this ^{64}Cu complex is taken up by breast cancer cells and is a substrate for Pgp, but that the biological properties are less than optimal. This optimization is the current objective of this project.

The U.S. Army Medical Research and Materiel Command under DAMD17-99-1-9125 supported this work.